Suppression of Root-Knot Nematode by Vermicompost Tea Prepared From Different Curing Ages of Vermicompost

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Abstract

Suppression of root-knot nematodes (*Meloidogyne* spp.) by vermicompost tea (VCT) has been inconsistent. Greenhouse and laboratory trials were conducted to compare the effects of VCT prepared from different curing ages of vermicompost (VC) on root penetration, reproduction, and hatching of *M. incognita*. In the penetration experiment, zucchini (*Cucurbita pepo*) seedlings were drenched with VCT prepared from (i) uncured (UVC), (ii) partially cured (PVC), (iii) completely cured (CVC) vermicompost, and (iv) water or no vermicompost (NVC) 3 days prior to *M. incognita* inoculation. The experiment was repeated twice on cucumber (*Cucumis sativus*) and terminated one week after nematode

inoculation. All three trials showed that UVC and PVC reduced ($P \leq 0.05$) penetration of *M. incognita* compared with CVC and NVC. Two greenhouse trials showed that VCT from different curing ages of VC did not reduce the abundance of *M. incognita* juveniles in soil and eggs in roots 2.5 months after nematode inoculation. Two laboratory trials to examine hatching consistently showed that VCT from UVC and PVC suppressed hatching ($P \leq 0.05$) compared with NVC, achieving 83.1% hatch reduction by UVC. Overall, VCT from UVC and PVC suppressed root penetration and hatching, but not the reproduction of *M. incognita* over time.

Plant-parasitic nematodes are economically damaging pests of many vegetable crops, of which the root-knot nematodes, *Meloidogyne* spp., are the most destructive species in the tropics and subtropics (Perry et al. 2009). Sikora and Fernandez (2005) reported that root-knot nematode cause up to 33% yield loss on melon (*Cucumis melo*), 38% on tomato (*Solanum lycopersicum*), 25% on potato (*Solanum tuberosum*), and 20% on eggplant (*Solanum melongena*). In light of the increasing demand for sustainable and organic farming, crop producers are exploring the potential of organic amendments for plant-parasitic nematode management (Kwok et al. 1992; Oka 2010).

Vermicompost (VC) is a composting product of accelerated biodegradation of organic matter by earthworms and its associated microbes through nonthermophilic decomposition (Arancon et al. 2004; Atiyeh et al. 2000; Dominguez et al. 1997; Edwards and Burrows 1988). VC generally has higher concentrations of plant-available nutrients (NO_3 , exchangeable Ca, P, and soluble K) and significantly larger and more diverse microbial populations than regular thermophilic compost (Tognetti et al. 2005). Vermicompost tea (VCT) is an aqueous extract of VC containing numerous bioactive molecules as well as microbial populations derived from farm wastes (Edwards et al. 2006). VCT can be prepared through aerated or nonaerated processes. During the aeration process, water extractable mineral nutrients, biologically active metabolites such as humic acid, as well as plant growth regulators from the VC are extracted into VCT and microbial biomass is augmented due to higher oxygen content (Arancon et al. 2007; Edwards et al. 2006; Pant et al. 2009). No comparison had been made between aerated and nonaerated VCT for plant pathogen suppression. However, research comparing effects of aerated and nonaerated compost tea of compost other than vermicompost on plant disease suppression has been studied. Some research showed that aerated and nonaerated compost have similar effects on disease suppression against bacterial spot (Al-Dahmani et al. 2003), early blight on tomato and purple blight on onion (Haggag and Saber 2007), gray mold on geranium (Scheuerell and Mahaffee 2006), and damping off of

cucumber seedlings caused by *Pythium ultimum* if the aerated compost tea was supplemented with kelp and humic acid (Scheuerell and Mahaffee 2004). On the other hand, Cronin et al. (1996) reported that nonaerated compost tea of spent mushroom compost was more effective in plant disease suppression in contrast to aerated compost tea. The aim of the current research was not to compare aerated and nonaerated VCT, but to investigate the effects of aerated VCT in suppressing root-knot nematode. Aerated VCT was used for this study, as aerated compost tea has an advantage of shorter preparation time (1 to 2 days) and resulted in less putrescent odor, whereas nonaerated compost tea requires 1 to 2 weeks steeping time (Ingham 2005). The risk of contamination by human pathogens including *E. coli* is also lower under aerobic conditions (Kannangara et al. 2006).

Several popular press reports have indicated that VC and VCT suppressed plant-parasitic nematodes in different vegetable crops (Arancon et al. 2002; Edwards et al. 2007). However, Wang et al. (2014) showed that drenching with VCT prepared from cured VC only suppressed the abundance of reniform nematodes at the initial stage of a zucchini (*Cucurbita pepo*) crop but not toward the end of the crop. The use of VC has shown suppression of disease caused by fungal plant pathogens (Chaoui et al. 2002; Szczech 1999). However, its use against plant-parasitic nematodes needs further investigation.

Several factors could affect the performance of VCT on nematode suppression: (i) curing age of VC; (ii) frequency of VCT drenching; (iii) feed stock to prepare VC; and (iv) virulence of the target pests. This project focused on evaluating nematode suppressive effects of VCT affected by its curing age. Curing of VC is the separation of vermicompost from earthworms (*Eisenia fetida* and *Perionyx excavatus*) and allowing its nutrients to be mineralized into plant-available nutrients for 8 to 10 weeks in a cool, dark, partially vented, but relatively humid place (Pant and Wang 2014). This research is based on a hypothesis that uncured VC would support more beneficial microbes than cured VC and could reduce root-knot nematode infections on plants. Widmer et al. (1998) showed that the suppressive effect of fresh compost on colony growth of *Phytophthora nicotianae* was lost when composted municipal waste was cured for more than 3 months. Similarly, Danon et al. (2007) also demonstrated that suppressive effects of matured thermophilic compost prepared from sewage and yard waste against *Sclerotium rolfsii* was lost after one year of curing. In both examples, reduction in abundance of antagonistic microorganisms are responsible for the loss of pathogen-suppressive effects of these composts (Danon et al. 2007; Widmer et al. 1998). Specific objectives

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of this research were to compare the effects of aerated VCT prepared from different curing ages of VC on (i) root penetration, (ii) reproduction rate, and (iii) hatching of *Meloidogyne incognita*.

Materials and Methods

Vermicompost tea preparation. Vermicompost was initiated in a 1.8-m-long × 0.6-m-wide wooden bin layered with plastic tarp at Poamoho Experiment Station in March 2013 with approximately 100 g of commercial mix of red wiggler (*Eisenia fetida*) and blue worms (*Perionyx excavatus*) (Waikiki Worms Co., Honolulu, HI). The worms were fed weekly with farm waste such as lettuce (*Lactuca sativa*), kale (*Brassica oleracea* var. *sabellica*), papaya (*Carica papaya*), and banana (*Musa* spp.) peel. Vermicompost used for the experiment was collected fresh from the bin prior to each experiment. No earthworms were included in the VC harvested. This VC collected either (i) remained uncured (VC freshly separated from earthworms), (ii) was partially cured (cured for 1 to 1.5 months), or (iii) was completely cured (cured for >3 months). For curing, fresh VC separated from earthworms was kept in 24 × 24 × 8-cm³ plastic containers punched with holes on the lid to allow air movement. Vermicompost tea (VCT) was prepared by soaking each type of VC in water at 1:10 (v/v) ratio, and aerated for 24 h using 2.5 W Elite 800 air pumps (Rolf C. Hagen Inc., Montreal, Canada). VCT was filtered using a kitchen strainer to separate the solid from the liquid prior to application.

Nematode inoculum. *Meloidogyne incognita* eggs were obtained from 'Orange Pixie' tomato (*Solanum lycopersicum*) or coleus (*Plectranthus scutellarioides*) roots, where the nematodes were cultured under greenhouse conditions with sterile sand and soil mix for more than 3 months. Eggs were extracted using NaOCl and centrifugal flotation methods (Hussey and Barker 1973). Eggs extracted were placed on 60.3-μm mesh screen to allow egg hatch for 2 weeks. Hatched juveniles were then counted and used as inoculum.

Root penetration experiment. One greenhouse and two laboratory trials were conducted to compare effects of VCT prepared from different ages of VC on root penetration of *M. incognita* through root drenching. Trial I was conducted on raised benches in Gilmore Greenhouse at the University of Hawaii at Manoa, Honolulu, HI, with an average ambient temperature of 23.8°C. Two-week-old zucchini squash 'Felix' F1 (*Cucurbita pepo*) (Harris Seeds, Rochester, NY) seedlings were established in 1:1 (v/v) sterile sand:soil mix in 8.5-cm-tall × 7.2-cm-diameter styrofoam cups prior to initiation of the experiment. Plants were either drenched with VCT prepared from (i) uncured (UVC), (ii) partially cured (PVC), (iii) completely cured (CVC) VC, or (iv) drenched with water (NVC) at 15 ml/plant. The experiment was arranged in completely randomized design (CRD) with 4 replications. Two days after VCT drenching, each seedling was inoculated with 200 s-stage juveniles (J2s) of *M. incognita* delivered in 1 ml of water by pipette. One week after nematode inoculation, roots were removed from each pot and stained with acid fuchsin (Byrd et al. 1983) to check for root penetration by *M. incognita*.

The experiment was repeated in the laboratory (Trials II and III) except that 'Bush Champion' cucumber (*Cucumis sativus*) (W. Atlee Burpee and Co., Warminster, PA) was used as the test crop. Treatments were arranged in CRD with 5 replications. Cucumber seeds were directly seeded into 60-ml plastic cups containing 1:1 (v/v) sterile sand:soil mix. Cucumber seedlings were grown and maintained at room temperature (22.8°C) and drenched with VCT or water as described in Trial I at 10 days after seeding. Two days after VCT drenching, 200 J2s of *M. incognita* were inoculated into each cup. One week after nematode inoculation, roots were removed from each pot and stained with acid fuchsin (Byrd et al. 1983) to check for root penetration by *M. incognita*.

Reproduction experiment. Two greenhouse trials were conducted to compare effects of VCT prepared from different curing ages of VC on reproduction of *M. incognita*. One and a half-month-old 'Orange Pixie' tomato (*Solanum lycopersicum*) seedlings were transplanted into 20-cm-diameter clay pots containing sterile sand:soil (1:1 v/v) mix at the Magoon Greenhouse Facility, University of Hawaii at Manoa. Tomato seedlings were drenched with VCT prepared from (i) UVC, (ii) PVC, (iii) CVC, or (iv) NVC at 50 ml/plant two days after

transplanting. The experiment was arranged in CRD with 3 replications. Vermicompost tea was prepared using a similar protocol as described earlier. Two days after VCT drenching, each seedling was inoculated with 750 J2s of *M. incognita*. Drenching with VCT was repeated at two-week intervals at 100 ml/plant for 1.5 months. At 1.5 months after the initiation of drenching, tomato plants began to flower and fruit, so the VCT drenching was halted. Tomato plants were allowed to grow for another month after the last VCT drenching (50 ml VCT/plant) and the experiment was terminated.

The reproduction test was repeated once much as described above except that cucumber was used instead of tomato and four replications were used per treatment. 'Bush Champion' cucumber (W. Atlee Burpee and Co.) seeds were directly seeded into plastic pots (14-cm-diameter and 22-cm-tall) at Gilmore Greenhouse, University of Hawaii at Manoa, with an average temperature of 23.8°C. Each cucumber seedling was inoculated with 500 J2s of *M. incognita*. Cucumber seedlings were drenched at 2-week intervals at 50 ml VCT/plant for the first 1.5 months, and 100 ml VCT/plant for the following month. Similar to the tomato trial, this trial was also terminated at 2.5 months after the initiation of VCT drenching, except that the drenching was continued toward the end of the trial instead of halting at flowering stage.

A 250 cm³ soil subsample from each pot was collected at the termination of both trials. Nematodes were extracted using elutriation and centrifugal flotation method (Jenkins 1964; Byrd et al. 1976). Eggs of *M. incognita* were extracted from roots of tomato and cucumber plants using 0.6% NaOCl and centrifugal flotation methods (Hussey and Barker 1973). Juveniles of *M. incognita* and eggs were counted with an inverted microscope (Leica DM IL LED, Wetzlar, Germany). In addition, a 0-to-10-scale root-gall index (Netscher and Sikora 1990) and root and shoot weights were recorded in the second reproduction trial.

Hatching experiment. A laboratory trial was conducted to compare the effect of VCT prepared from different curing ages of VC on hatching of *M. incognita* eggs. *Meloidogyne incognita* eggs were extracted from 'Orange Pixie' tomato culture maintained in greenhouse using NaOCl and centrifugal flotation methods (Hussey and Barker 1973). VCT prepared from (i) UVC, (ii) PVC, (iii) CVC, and (iv) NVC were added into 60-ml plastic cups at 15 ml/cup. The experiment was maintained at 22.8°C and arranged in CRD with four replications. Each plastic cup served as a hatching chamber where 200 freshly extracted *M. incognita* eggs suspended in 1 ml of water was placed on a screen of 60.3-μm pore size in the middle of the cup (Fig. 1). This mesh size kept nematode eggs on the screen but allowed J2s to pass through. VCT or water was replaced every day with fresh VCT or water to avoid bacterial build-up and development of anaerobic conditions. Hatched J2s were collected every day for 15 days and total hatching was counted with an inverted microscope (Leica DM IL LED, Wetzlar, Germany). The egg hatching experiment was repeated once.

Statistical analysis. All data were subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure in Statistical Analysis System (SAS Institute Inc., Cary, NC). Prior to ANOVA, nematode data were log transformed [$\log_{10}(x+1)$] wherever appropriate to normalize the data distribution.

Fig. 1. Nematode eggs placed in a hatching chamber containing a 60.3-μm mesh screen inside a 60-ml plastic cup incubated in VCT or water.

Untransformed means are presented. Means were separated using Waller-Duncan k -ratio ($k = 100$) t test wherever appropriate.

Results

Root penetration experiment. Based on results from Trial I and Trial II, VCT from UVC and PVC suppressed root penetration of *M. incognita* ($P \leq 0.05$) compared with NVC, whereas VCT from CVC did not suppress the nematode penetration ($P > 0.05$; Fig. 2). In Trial III, VCT from all UVC, PVC, and CVC suppressed root penetration of *M. incognita* ($P \leq 0.05$; Fig. 2) compared with NVC. However, VCT prepared from UVC and PVC further reduced 78% of nematode penetration as compared with that prepared from CVC. Reduction of *M. incognita* root penetration was not due to effects of VCT on root weight as similar root weights were observed among treatments in all three trials ($P > 0.05$, data not shown).

Reproduction experiment. No difference in numbers of *M. incognita* extracted from the soil and eggs extracted from the roots was observed among treatments in both Trials ($P > 0.05$, data not shown). Root gall index of tomato in all treatments were rated similar at an index of 8 with no significant reduction in root galling by VCT ($P > 0.05$, data not shown). All tomato plants were severely stunted at 2.5 months after initiation of drenching. Thus, shoot and root weights in the tomato trial were not recorded. Shoot and root weights, and root gall index of cucumber plants in the second trial were also not affected by VCT drenching ($P > 0.05$; Fig. 3).

Hatching experiment. In the first trial, numbers of egg hatched was significantly lower in VCT prepared from all three ages of VC as compared with that in the water control ($P \leq 0.05$). However, in the second trial, only VCT from UVC and PVC ($P \leq 0.05$) but not CVC ($P > 0.05$) reduced the hatch of *M. incognita* as compared with the water control (Fig. 4).

Discussion

Results from the root penetration and hatching experiments supported the hypothesis that VCT prepared from UVC suppressed *M. incognita* more effectively and consistently than VCT prepared from CVC. This result was more obvious in Trial III, possibly due to higher *M. incognita* viability in Trial III than in Trials I and II, resulting in more nematode penetration in the NVC. This result was similar to results using other thermophilic compost to suppress various fungal pathogens, as the suppressive effect of fresh compost was lost after a

Fig. 4. Effect of vermicompost tea (VCT) prepared from completely cured (CVC), partially cured (PVC), and uncured (UVC) vermicompost compared with water or no vermicompost (NVC) on hatching of *M. incognita* over 15 days of incubation in two trials. Means ($n = 4$) labeled with same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t test.

Fig. 2. Effect of vermicompost tea (VCT) on numbers of *M. incognita* penetrated per plant on zucchini (Trial I) and cucumber (Trial II and III) 7 days after nematode inoculation. Plants were drenched with VCT prepared from completely cured (CVC), partially cured (PVC), uncured (UVC) vermicompost, or no vermicompost (NVC). Means are average of 4 replications in Trial I and 5 replications in Trial II and Trial III. Nematode abundance data were log transformed, $\log(x+1)$, prior to analysis of variance. Bars labeled with same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t test.

Fig. 3. Effect of vermicompost tea (VCT) prepared from completely cured (CVC), partially cured (PVC), and uncured (UVC) vermicompost compared with water or no vermicompost (NVC) on A, shoot and root weight, and B, root gall index of the cucumber. Means ($n = 4$) labeled with same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t test.

curing or storage period (Widmer et al. 1998; Danon et al. 2007). This result also demonstrated that the reduction in root penetration of *M. incognita* by VCT was not due to its effects on root growth. Although Pant et al. (2009) reported that drenching VCT from cured VC enhanced pak choi (*Brassica rapa* var. *chinensis*) growth and yield, enhancement of plant growth was not apparent in this short-term experiment. The possible mechanism by which VCT could suppress root penetration or hatching of *M. incognita* could be due to (i) induction of host-plant resistance by beneficial rhizobacteria, (ii) secretion of hydrolytic enzymes such as chitinase that cause hydrolysis of nematode eggs or protease that hydrolyze the proteins protecting the chitin layer of nematode egg shell thus exposing the chitin layer to chitinase, or (iii) association with bacteria that exert competitive exclusion or microbial antagonistic effects against the nematodes.

Several reports have documented more diverse and higher bacterial activities in soil treated with VCT than without VCT (Elmer 2009; Gopal et al. 2009; Vaz-Moreira et al. 2008). Some of these bacteria commonly found in VC include *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Azobacter*, *Azospirillum*, *Enterobacter*, and *Nitrobacter* (Pathma and Sakthivel 2013). Less effective nematode suppression in CVC than that in PVC and UVC in this study could be related to the activities of rhizobacteria such as chitinolytic activities that might have ability to suppress egg hatch of *M. incognita*. Jung et al. (2002) reported that the chitinase-producing *Paenibacillus illinoisensis* suppressed hatching of *M. incognita*. However, the theoretical difference in the abundance of rhizobacteria from VCT prepared from different curing ages of VC remains to be tested.

Unfortunately, suppression of *M. incognita* root penetration by VCT did not lead to the suppression of *M. incognita* reproduction and root gall formation over time in the reproduction experiment. The *M. incognita* inoculum used in the reproduction experiment was J2s, thus enzymatic effect of VCT against hatching would not be able to suppress *M. incognita*. Another mechanism of the suppression of *M. incognita* by VCT against J2s infection is the induction of host-plant resistance. Induction of host-plant resistance by VCT could be affected by frequency of VCT drenching. Drenching at 2- or 4-week intervals in the reproduction experiment might not be frequent enough for VCT to induce host-plant resistance continuously.

In conclusion, the findings from this study clearly demonstrated that VCT prepared from uncured VC suppressed *M. incognita* root penetration better than that prepared from cured VC. Information on the definitive mode of action for VCT to control plant-parasitic nematodes is still limited. Further studies are in progress to investigate if VCT could induce host-plant resistance against *M. incognita*, and to determine effective drenching intervals of VCT to suppress reproduction of *M. incognita*.

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